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# A Study on the Nutrient Content, Phytochemicals and Antioxidant Potential of Raw, Germinated and Fermented *Moringa Oleifera* Seeds

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**Abstract:** Diet and Nutrition are important factors in the promotion and maintenance of good health throughout the life. A plant-based diet can prevent and treat chronic diseases. Health is “Absence of Disease”. *Moringa oleifera* is rich in macro and micronutrients like protein, carbohydrate, calcium, phosphorus, iron, vitamins, beta carotene and other bioactive compounds which are important for normal functioning of the body, prevention and curing of certain diseases. *M. oleifera* has excellent therapeutic properties including anticancer, antiulcer, antimicrobial, antioxidant. Various researches have concluded that *Moringa* should be used as a functional ingredient in food products. This study was taken up to investigate the effects of germination and fermentation on the nutritional, bioactive, and antimicrobial characteristics of moringa seed and it is found to have essential phytochemicals, micro and macro nutrients which can be used as natural remedies to prevent various diseases and deficiencies.

**Keywords:** *Moringa Oleifera* Seeds, Germinated, Fermented, Nutrient, Phytochemicals, Antioxidant Potential

## I. INTRODUCTION

Diet and nutrition are important factors in the promotion and maintenance of good health throughout the life. Nowadays, with continuously changing socio-economic status, people have become more concerned about their health [1]. Mankind has always been interested in food. Several studies over the last 50 years have clearly shown that diets dominated plant-based foods such as fruits, vegetables and dietary fibres prevent and reduce the risk of chronic diseases such as cardiovascular diseases, obesity, diabetes, and so on and promote human health [2]. The generation of scientific research linking foods of plant origin and health worldwide has resulted that plant bioactive compounds have antioxidant and other healthy properties [3]. Ayurveda and traditional medicine have utilized natural resources such as plants and trees as part of their regime to treat different illness and diseases with positive outcomes. As an effect of growth in population and agricultural expansion, only a few forests are remaining, and they are highly degraded, causing losses in traditionally important nutritious foods, medicines and other useful products leading to food insecurity [4].

*Moringa oleifera* (Moringaceae) is one tree having enormous nutritional and medicinal benefits [1]. Nearly all parts have shown to be effective against several ailments including cancer which was attributed to the bioactive constituents [5]. *M. oleifera* is commonly known as the drumstick tree or horse radish tree [6]. It is used as a vegetable and also in Indian folk medicine for the treatment of various illnesses. The length of plant can reach 12m with soft and brittle stems. The diameter is about 20-40 cm and lifespan are about 30 years. Moringa leaves are compound, pinnate double, and of small round or oval shape. The fruit, called “drumstick,” is long and angular, its sides form a triangle; the drumsticks are about 15-45 cm long [7]. Taller plants bear cream-white, small size flowers in clusters throughout the season, which subsequently develop into slender dark-green, three-sided, edible seed pods with tapering ends. Each pod contains 20 fleshy pulp and round pea-sized seed encased inside a wing shaped coat (hull).

*M. oleifera*, native to sub-Himalayan Mountain of Northern India grows in the tropical and subtropical areas of the world. *M. oleifera*, the native of sub-Himalayan Mountain of Northern India; is now cultivated for a variety of purposes in the whole tropical and sub-tropical regions of the world [8]. India is one of the large producers of moringa, with an annual production of 1.1 to 1.3 million tons of fruits from an area of 380 km<sup>2</sup> [9]. The cultivation of moringa in India occurs mainly in the southern states of Tamil Nadu, Karnataka, Kerala, and Andhra Pradesh. In India, Andhra Pradesh stands at the first position in both area and production followed by Karnataka and Tamil Nadu. Moringa is commonly found in countries of Southeast Asia, the Philippines, the Middle East, Africa, and the Caribbean.

*M. oleifera* is an extremely valued plant, which has an effective range of medicinal uses in traditional folk medicine with high nutritional value. Many pharmacological researches have shown the ability of this plant to exhibit analgesic, anti-inflammatory, antipyretic, anticancer, antioxidant, nootropic, hepatoprotective, gastro protective, anti-ulcer, cardiovascular, anti-obesity, antiepileptic, anti-asthmatic, anti-diabetic, anti-urolithiasis, diuretic, local anaesthetic, anti-allergic, anthelmintic, wound healing, antimicrobial, immune-modulatory, and anti-diarrheal properties [9].

Deficiencies in micronutrients across the globe can be prevented or eliminated if adequate nutrients included in the diet [10]. *M. oleifera* is rich in macro and micronutrients like protein, carbohydrate, calcium, phosphorus, phosphorus, iron, vitamins, beta carotene and other bioactive compounds which are important for normal functioning of the body and prevention and curing of certain diseases [1]. Evidence defines clear and concrete information on the benefits of moringa seeds regarding human consumption, which is beneficial to human health. *M. oleifera* seed contain a significant amount of oil with a high-quality fatty acid thus Moringa oil could be a good substitute for olive oil in the diet. Our aim was to germinate and ferment moringa seeds to compare the benefits with the raw seeds.

According to our knowledge, there is not much comparative study done on raw, germinated and fermented *M. oleifera* seeds. Therefore, the present study on the chemical and nutritional composition of raw, germinated, fermented *M. oleifera* seeds, phytochemical content and their potential uses for human health which would be helpful to the mankind in many ways. Moringa is globally available plant and the seeds are a promising resource for food and non-food applications,

## II. MATERIALS AND METHODS

The Family, genus and Species of *M. oleifera* was identified in the Plant Biotechnology Department, Women’s Christian College, Chennai. Seeds of *M. oleifera* were collected from the market at Madhavaram, Chennai, Tamil Nadu. Raw *M. oleifera* seeds were sorted, rinsed, sundried, dehulled and milled using a mechanical blender. The sample is sieved to obtain raw *M. oleifera* (RMO) seed flour.

### A. Germinated *M. oleifera* Seed (GMO) Flour

*M. oleifera* seeds were sorted and soaked in potable water for 2 hours. Excess water was removed and the seeds were tied in a wet cloth for 72 hours (3 days) at room temperature. Germinated Seeds were selected and washed thoroughly, sundried, dehulled and milled using a mechanical blender and sieved to obtain germinated *M. oleifera* (GMO) seed flour.

### B. Fermented *M. oleifera* Seed (FMO) Flour

*M. oleifera* seeds were sorted, dehulled [11], and soaked in water for 72 hours (3 days) to ferment. The fermented seeds were sundried and milled using a mechanical blender and sieved to obtain fermented *M. oleifera* (FMO) seed flour.

All the three samples were packed in a plastic container sealed and stored at room temperature prior to analyses.

### C. Preparation of Extract

About 20 g of each sample was soaked in 100ml of ethanol for 72 h. The pale-yellow supernatant liquid was filtered by Whatman filter paper. The extract was condensed in a hot plate at 50°C, which yields gummy extract.

Qualitative and quantitative analysis of phytochemicals, identification of volatile bioactive compounds using gas chromatography mass spectrometry (GC-MS), estimation of macro nutrients and selected micro nutrients and the antioxidant potential of the samples was estimated with the condensed sample. Table I gives the details regarding the analysis phytochemicals.

Table I. Details Quantitative Analysis Of Phytochemicals

| Sn.No | Compound           | Name of test         | Observation                                      | Interpretation |
|-------|--------------------|----------------------|--|----------------|
| 1     | carbohydrates      | Fehling’s test       | A brick red precipitate                          | Presence       |
| 2     |                    | Molish test          | Reddish brown ring at the junction of two layers | Presence       |
| 3     | Alkaloids          | Mayer’s test         | creamy white precipitate                         | Presence [12]  |
| 4     |                    | Hager’s test         | A prominent yellow precipitate                   | Presence [13]  |
| 5     | glycosides         | Borntrager’s test    | Pink color                                       | Presence [12]  |
| 6     | Saponins           | Foam test            | appearance of turbid foam                        | Presence [13]  |
| 7     | phenolic compounds | Ferric chloride test | A dark green or blue colour                      | Presence [14]  |
| 8     |                    | Flavonoids           | white precipitate                                | Presence       |
| 9     | Terpenoids         | Salkowski test       | A reddish-brown coloration                       | Presence       |

### III.RESULTS

#### A. Qualitative Assessment of Phytochemicals in RMO, GMO and FMO Seeds

Phytochemical screening was carried out for *in RMO, GMO and FMO* seeds to identify the presence and absence of major natural chemical groups such Carbohydrate, Protein, Alkaloids, Flavonoids, Saponins, Terpenoids, Tannins, Phenols, Steroids.

TABLE II  
QUALITATIVE RESULTS FOR VARIOUS PHYTOCHEMICALS

| S. No | Phytochemical test | RMO      | GMO      | FMO      |
|-------|--------------------|----------|----------|----------|
| 1     | Carbohydrate test  | Positive | Positive | Positive |
| 2     | Protein test       | Positive | Positive | Positive |
| 3     | Alkaloid test      | Positive | Positive | Positive |
| 4     | Flavonoid test     | Positive | Positive | Positive |
| 5     | Saponin test       | Positive | Positive | Positive |
| 6     | Terpenoid test     | Positive | Positive | Positive |
| 7     | Tannin test        | Positive | Positive | Positive |
| 8     | Phenol test        | Positive | Positive | Positive |
| 9     | Steroid test       | Positive | Positive | Positive |

- Presence of phytochemicals (Positive)
- Absence of phytochemicals (Negative)

#### B. Quantification of Phytochemicals in in RMO, GMO and FMO Seeds

Table III shows the total the total flavonoid content in 100 grams of RMO, GMO and FMO is 129.37, 326.36 and 268.4 µg QE/g respectively. 221.00, 464.19 and 339.14 mg/g gallic acid equivalent phenols is present in 100 grams of each sample.

TABLE III  
Estimation Of Total Phenol And Flavonoids Content in *in RMO, GMO AND FMO SEEDS*

| S. No | Phytochemical                                       | Quantity Present |        |        |
|-------|---|------------------|--------|--------|
|       |   | RMO              | GMO    | FMO    |
| 1     | Phenols<br>(mg/g gallic acid equivalent)            | 221.00           | 464.19 | 339.14 |
| 2     | Flavonoids<br>(µg QE/g) QE-Quercetin<br>Equivalent) | 129.37           | 326.36 | 268.4  |

#### C. Identification of volatile bioactive compounds using Gas Chromatography Mass Spectrometry (GC-MS) in in RMO, GMO and FMO Seeds

The interpretation of GC – MS was elucidated using National Institute Standard and Technique (NIST). The particular compounds present in the sample were identified by matching their mass spectral fragmentation patterns of the respective peaks in the chromatogram with those stored In the National Institute Standard and Technology Mass spectral database library. The spectrum of the unknown components was compared with the spectrum of the known components stored in NIST library.

##### 1) Volatile Bioactive Constituents Identified in RMO Seeds Using GC – MS

The following table IV presents the compounds that were identified by comparing the spectrum of the unknown components with that of the spectrum of the known compounds stored in the NIST library. Eight volatile bioactive components were identified in RMO. The compound name molecular formula, Structure, Molecular mass and the property are presented in table IV.

According to table IV the predominant volatile constituents in the RMO seeds are: Ethyl 2-quinoxalinecarboxylate, Methyl tetradecanoate, Pentadecanoic acid methyl ester, 1H-indene, 2-decyloctahydro, Hexadecanoic acid methyl ester, 8 – octadecenoic acid methyl ester, Z, Z-4,16-Octadecadien-1-ol acetate, Methyl eicosa-5,8,11,14,17 pentaenoate.



TABLE IV

Compounds Identified In Rmo Seeds Through Gas Chromatography – Mass Spectrometry Components That Eluted In The Chromatogram In The Increasing Order Of Polarity

| S.No | Peak | Formulae  | Compound name                           | Molecular Mass (g/mol) | Property  |
|------|------|---|---|------------------------|---|
| 1    | 13.7 | C <sub>11</sub> H <sub>10</sub> N <sub>2</sub> O <sub>2</sub> | Ethyl 2-quinoxalinecarboxylate          | 201.3                  | Antipsoriatic, Antineoplastic                                 |
| 2    | 15.0 | C <sub>15</sub> H <sub>30</sub> O <sub>2</sub>                | Methyl tetradecanoate                   | 242.4                  | Not available   |
| 3    | 15.7 | C <sub>16</sub> H <sub>32</sub> O <sub>2</sub>                | Pentadecanoic acid, methyl ester        | 256.4                  | Antiacne agent, Antipsoriatic, Antiviral, Antioxidant         |
| 4    | 16.6 | C <sub>19</sub> H <sub>36</sub>                               | 1H-indene, 2-decyloctahydro             | 264.0                  | Not available   |
| 5    | 17.2 | C <sub>17</sub> H <sub>34</sub> O <sub>2</sub>                | Hexadecanoic acid methyl ester          | 270.0                  | Antiasthmatic, antidepressant, antibacterial, antiviral Agent |
| 6    | 18.9 | C <sub>19</sub> H <sub>36</sub> O <sub>2</sub>                | 8 – octadecenoic acid, methyl ester     | 296.0                  | Antioxidant   |
| 7    | 21.1 | C <sub>20</sub> H <sub>36</sub> O <sub>2</sub>                | Z, Z-4,16-Octadecadien-1-ol acetate     | 308.0                  | Not available   |
| 8    | 22.3 | C <sub>21</sub> H <sub>32</sub> O <sub>2</sub>                | Methyl eicosa-5,8,11,14,17- pentaenoate | 316.0                  | Antipsoriatic, Antineoplastic, Anti-allergic                  |

2) Volatile Bioactive Constituents Identified in GMO Seeds Using GC – MS

According to table V, the predominant volatile constituents in the GMO seeds are Undecanoic acid, 3-Methyl-5-(1, 4, 4-trimethylcyclohex-2-enyl) pentan-1-ol), Methaqualone, 9-Hexadecenoic acid, Hexadecanoic acid, methyl ester, Hexadecanoic acid, ethyl ester, 8-octadecenoic acid, methyl ester, Benzimidazole. Three compounds eluted are 9-Hexadecenoic acid of molecular mass 268.00 g/mol, Hexadecanoic acid, methyl ester of molecular mass 270.00 g/mol and 8- octadecenoic acid, methyl ester of molecular mass 269.00 g/mol.

TABLE V

Compounds Identified In Gmo Seeds Through Gas Chromatography – Mass Spectrometry Components That Eluted In The Chromatogram In The Increasing Order Of Polarity

| S. No | Peak | Compound name   | Formula  | Molecular mass (g/mol) | Property  |
|-------|------|---|--|------------------------|---|
| 1     | 13.1 | Undecanoic acid   | C <sub>11</sub> H <sub>22</sub> O <sub>2</sub>   | 200.0                  | Antioxidant   |
| 2     | 14.1 | 3-Methyl-5-(1,4,4-trimethylcyclohex-2-enyl) pentan-1- ol) | C <sub>13</sub> H <sub>20</sub> O                | 224.0                  | Antioxidant   |
| 3     | 14.6 | Methaqualone  | C <sub>16</sub> H <sub>14</sub> N <sub>2</sub> O | 250.0                  | Antibacterial, Anti Allergic                                  |
| 4     | 15   | 9-Hexadecenoic acid                                       | C <sub>16</sub> H <sub>30</sub> O <sub>2</sub>   | 268.0                  | Antioxidant, Anticonvulsant, Antibacterial Agents             |
| 5     | 15.2 | Hexadecanoic acid, methyl ester                           | C <sub>17</sub> H <sub>34</sub> O <sub>2</sub>   | 270.0                  | Antiasthmatic, Antidepressant, Antibacterial, Antiviral Agent |
| 6     | 15.9 | Hexadecanoic acid ethyl ester                             | C <sub>18</sub> H <sub>36</sub> O <sub>2</sub>   | 284.0                  | Antidepressant, Antibacterial                                 |
| 7     | 16.9 | 8-octadecenoic acid, methyl ester                         | C <sub>19</sub> H <sub>36</sub> O <sub>2</sub>   | 269.0                  | Antioxidant   |
| 8     | 17.9 | Benzimidazole   | C <sub>7</sub> H <sub>6</sub> N <sub>2</sub>     | 308.0                  | Anti – Infective  |

3) Volatile Bioactive Constituents Identified in FMO Seeds Using GC – MS

from table VI, the predominant volatile constituents in the *FMO Seeds* are: Vinyl lauryl ether, 5-Octadecene, Benzoic acid, 3,5-bis (1,1-dimethylethyl)-4- hydroxy, 9-Hexadecenoic acid, Hexadecanoic acid, 3-Eicosene, 9-Octadecenoic acid, 5- [4(Diethylamino) benzylideneamino] benzimidazol-2 (3H) one, 1-Tetradecene, 2-decyl. Three compounds peaked eluted are 9-Hexadecenoic acid of molecular mass 268.00 g/mol, Hexadecanoic acid, methyl ester of molecular mass 270.00 g/mol and 9- Octadecenoic acid, of molecular mass 296.00 g/mol.

TABLE VI

Compounds Identified In Fmo Seeds Through Gas Chromatography – Mass Spectrometry Components That Eluted In The Chromatogram In The Increasing Order Of Polarity

| S. No | Peak | Compound name  | Formula      | Molecular mass (g/mol) | Property  |
|-------|------|--|--------------|------------------------|---|
| 1     | 13.0 | Vinyl lauryl ether   | C14H28O      | 212.0                  | Anti-infective, antiseptic                        |
| 2     | 13.7 | 5-Octadecene   | C18H36       | 212.0                  | Not available                                     |
| 3     | 14.6 | Benzoic acid 3,5-bis (1,1- dimethylethyl)-4-hydroxy          | C15H22O<br>3 | 250.0                  | Anti-asthmatics, anticonvulsants, antidepressants |
| 4     | 15   | 9-Hexadecenoic acid  | C16H30O<br>2 | 268.0                  | Antioxidant, antiseptic                           |
| 5     | 15.3 | Hexadecanoic acid  | C16H32O<br>2 | 270.0                  | Antidepressants, Antioxidant, Anti-infective      |
| 6     | 15.9 | 3-Eicosene   | C20H40       | 280.0                  | Not available                                     |
| 7     | 16.9 | 9-Octadecenoic acid  | C18H34O<br>2 | 296.0                  | Anti-Parkinson drugs                              |
| 8     | 17.9 | 5 [4(Diethylamino) benzylideneam ino] benzimidazol-2(3H) one | C7H6N2       | 308.0                  | Anti-asthmatics, Antioxidant                      |
| 9     | 19.6 | 1-Tetradecene, 2-decyl                                       | C24H48       | 336.0                  | Antioxidant                                       |

D. Estimation of Macro Nutrients and Selected Micro Nutrients in RMO, GMO and FMO Seeds

From table VII, it is clear that 100g of each RMO, GMO and FMO seeds contain 379.49 kcal, 349.45 kcal and 354.44 Kcal respectively. RMO provides more energy than GMO and FMO seeds. Also, it was observed that 100 grams of each RMO, GMO and FMO seeds provide 33.22, 49.13 and 29.28g of carbohydrate. GMO seeds provide more carbohydrates than RMO and FMO seeds. On top of this, GMO seeds have a higher amount of protein than RMO and FMO seeds. Finally, it is evident that 100 grams of each RMO, GMO and FMO seeds contain 36.46 g, 25.98g and 31.19g respectively. All three samples provide approximately the same amount of fat. The main energy source of *M. oleifera* seeds is from fat. Thus *M. oleifera* seeds are used for extracting oil for various uses.

From table VII, 100 grams of each RMO, GMO and FMO seeds contain 5.6 g, 5.4 g and 5.9 g crude fibre respectively. All three samples provide approximately the same amount of crude fibre. But out of the three, FMO seeds provide more crude fibre. Thus, it is evident that GMO and FMO seeds have more nutritional value than the RMO seeds. On top of that. GMO seeds provide more phosphorus, iron, Ascorbic acid and calcium. Every part of *M. oleifera* is a storehouse of important nutrients and anti-nutrients. The seeds of *M. oleifera* are rich in minerals like calcium, phosphorus, zinc, magnesium, iron and copper are essential for growth and development [15].

TABLE VII  
Quantification Of Macro Nutrients In RMO, GMO and FMO Seeds

| S. No | Nutrients          | Quantity present in 100g of sample |       |       |
|-------|--------------------|------------------------------------|-------|-------|
|       |                    | RMO                                | GMO   | FMO   |
| 1     | Energy (Kcal)      | 379.5                              | 349.4 | 354.4 |
| 2     | Carbohydrates (g)  | 33.2                               | 49.1  | 59.3  |
| 3     | Protein (g)        | 36.5                               | 56.6  | 31.1  |
| 4     | Fat (g)            | 37.0                               | 25.9  | 31.2  |
| 5     | Crude fiber (g)    | 5.6                                | 5.4   | 5.9   |
| 6     | Phosphorus (mg)    | 33.3                               | 34.2  | 32.8  |
| 7     | Calcium (mg)       | 132.2                              | 137.9 | 124.8 |
| 8     | Iron (mg)          | 7.4                                | 7.5   | 6.5   |
| 9     | Ascorbic acid (mg) | 24.8                               | 26.1  | 17.7  |

E. The Antioxidant Potential of RMO, GMO and FMO Seeds

Antioxidant has increased interest in order to prevent disastrous effects of the free radicals and prevent the deterioration of fats and other food items. In both cases, antioxidants have a natural preference compared to synthetic sources [16]. There is therefore a drastic increase in the use of methods for estimating the efficiency of such substances as antioxidants **Error! Reference source not found.**

1) Free Radical Activity of the RMO, GMO and FMO Seeds using DPPH Assay

In the present study, the ethanol extract of RMO, GMO and FMO Seeds was used to investigate the antioxidant activity by DPPH (2,2-diphenyl-1-picryl hydrazyl) method and FRAP assay based on the rapid reduction in ferric-tripyridyltriazine (Fe<sup>III</sup>-TPTZ) by antioxidants present in the samples forming ferrous-tripyridyltriazine (Fe<sup>II</sup>-TPTZ), a blue-coloured product [18].

TABLE VIII  
COMPARISON OF RADICAL SCAVENGING ACTIVITY OF RMO, GMO AND FMO WITH STANDARD ASCORBIC ACID USING DPPH ASSAY AND FRAP (Fe<sup>3+</sup>) ASSAY

| Concentration (µg/mL) | DPPH Assay |            |            | FRAP (Fe <sup>3+</sup> ) Assay |            |            |
|-----------------------|------------|------------|------------|--------------------------------|------------|------------|
|                       | RMO        | GMO        | FMO        | RMO                            | GMO        | FMO        |
|                       | Mean (SD)  | Mean (SD)  | Mean (SD)  | Mean (SD)                      | Mean (SD)  | Mean (SD)  |
| 50                    | 46.4 (1.2) | 62.5 (0.9) | 31.8 (1.2) | 22.5 (1.2)                     | 56.5 (1.2) | 48.5 (1.2) |
| 100                   | 56.3 (1.0) | 74.3 (0.6) | 42.6 (1.5) | 32.6 (0.3)                     | 71.9 (0.6) | 56.5 (0.6) |
| 150                   | 59.2 (0.7) | 86.1 (1.2) | 52.9 (0.7) | 41.8 (0.9)                     | 75.8 (0.9) | 73.6 (1.2) |
| 200                   | 63.8 (1.3) | 86.7 (0.8) | 65.6 (0.2) | 53.8 (1.0)                     | 85.3 (1.0) | 79.0 (0.7) |
| 250                   | 74.4 (1.5) | 87.9 (0.6) | 78.5 (1.0) | 70.6 (0.7)                     | 87.7 (1.7) | 82.6 (1.0) |
| 300                   | 83.6 (1.0) | 89.7 (1.0) | 88.4 (0.8) | 75.6 (1.0)                     | 90.4 (0.2) | 85.7 (0.7) |
| F Value               | 396.1      | 436.9      | 1174.1     | 1522.66                        | 419.15     | 790.45     |
| P Value               | 0.0001     | 0.0001     | 0.0001     | 0.0001                         | 0.0001     | 0.0001     |

From the table VIII, the radical scavenging activity of RMO, GMO and FMO seeds increase with the concentration. It is found that the P value for RMO, GMO and FMO is 0.0001 (p<0.01) which is significant at 1 per cent level. Thus, there is a significant difference between the RMO, GMO and FMO was comparable to the percentage of inhibition of the reference standard (ascorbic

acid) at all concentrations.

2) *IC50 (inhibition concentration 50) and RC50 (Reduction concentration at 50 %) in RMO, GMO and FMO Seeds*

IC50 (inhibition concentration 50) is the antioxidant concentration ( $\mu\text{g/mL}$ ) which is able to inhibit 50 per cent of the activity of free radicals. From the table 15, it is evident that GMO seed have the lowest IC50 value. 40.00  $\mu\text{g/mL}$  of the Moringa seed extract can have 50 per cent of inhibiting property. The IC50 value of RMO seed is 53.82  $\mu\text{g/ml}$  whereas the IC50 value of the FMO seed is 141.72  $\mu\text{g/mL}$  which is the highest. The samples when compared to the IC50 value of the standard ascorbic acid (2.9  $\mu\text{g/mL}$ ) RMO, GMO and FMO samples requires higher concentration to reduce 50 per cent of free radical

TABLE IX  
COMPARISON OF IC50 VALUE OF RMO, GMO AND FMO SEEDS

| SAMPLE                 | IC50 VALUE             | RC50 VALUE             |
|------------------------|------------------------|------------------------|
| RMO                    | 53.8 $\mu\text{g/MI}$  | 179.5 $\mu\text{g/mL}$ |
| GMO                    | 40.0 $\mu\text{g/mL}$  | 44.2 $\mu\text{g/mL}$  |
| FMO                    | 141.7 $\mu\text{g/mL}$ | 551.6 $\mu\text{g/mL}$ |
| STANDARD ASCORBIC ACID | 2.9 $\mu\text{g/mL}$   |                        |

RC50 (Reduction concentration at 50 %) is the antioxidant concentration ( $\mu\text{g/ml}$ ) which is able to reduce 50 per cent of the activity of free radicals. From the table, it is evident that compared to the RC50 value of the standard ascorbic acid (2.88  $\mu\text{g/mL}$ ) the test samples require a higher concentration to reduce 50 per cent of free radical. The RC50 value of Germinated Moringa seeds 44.21  $\mu\text{g/mL}$  which is very low compared to raw and fermented which is 179.46  $\mu\text{g/mL}$  and 551.56  $\mu\text{g/mL}$  respectively.

IV.DISCUSSION

Natural plant products have been on the increase leading to the identification and improvement of plant products beneficial to mankind. According to Bagchi [19] ‘Foods or dietary components that may provide a health benefit beyond basic nutrition,’ he also stated that functional foods are ‘Foods that by virtue of physiologically vital food elements provide health benefits beyond basic nutrition’. There is great scope for foods that can impart health benefits beyond traditional nutrients. Within more functional perspectives, health has been defined in terms of a certain quality of life which enables individuals to live happily, successfully, fruitfully, and creatively [20]. The current World Health Organization (WHO) definition of health, describes health as “a state of complete physical, mental and social well-being and not merely the absence of disease or infirmity.

Germination of seeds helps to add on to the nutrient content of the seeds, especially the amount of protein. Thus, Sprouts help to get protein from the least amount of seed consumption. Even though there are many other benefits to germinated seeds in food, more research must be done to improve texture and sensory properties to gain community acceptance [21].

Fermented foods are digested easily and reactive foods into health – giving foods. Fermentation destroys most of the harmful microorganisms and chemicals in foods and adds beneficial bacteria in the gut. The enzymes developed and bacteria grown during fermentation aids in the digestion of foods. But not all forms of fermentation or fermented foods are safe and nutritious. For example, certain microorganisms such as fungi can contaminate the foods and may enhance the production of N-nitroso compounds with potential carcinogenic properties [22]. Still fermenting seeds will help to increase the nutrient content and the probiotic concentration which have a lot of health benefits.

*M. oleifera* is a miracle plant in which all the parts of the plant are used for medicinal purposes. Leaves, pods, flower and seeds of *M. oleifera* are considered as a functional food. Root and bark acts as a cardiac stimulant, anti-ulcer and anti-inflammatory agent [23]. The root and bark contain minerals like calcium, magnesium and sodium and alkaloids like morphine, moriginine which helps the bark to be antiulcer and helps to relax the muscles [21]. The leaves of *M. oleifera* are rich in minerals like phosphorus , calcium, zinc, iron, magnesium and copper [15] and Vitamins like beta-carotene, vitamin B such as folic acid, pyridoxine and nicotinic acid, vitamin C, D and E [25]. Moringa leaves treat asthma, hyperglycaemia, Dyslipidaemia, flu, heart burn, syphilis, malaria, pneumonia, diarrhoea, headaches, scurvy, skin diseases, bronchitis, eye and ear infections [26] Also reduces, blood pressure and cholesterol and acts as an anticancer, antimicrobial, Antioxidant, anti-diabetic and anti- atherosclerotic agents, neuro-protectant [27]. The presence of minerals and vitamins help in boosting the immune system and cure a myriad of diseases [28]. Various amino acids and Phytochemicals provide anti-diabetic and antioxidant properties to the leaves.

Moringa seeds are rich in antioxidant thus it reduces the oxidative stress which is a risk factor to heart disease, heart failure, and



high blood pressure [29].

High cholesterol in the blood is a risk factor of heart disease. In traditional Thai medicine, Moringa seeds are used as a cardiogenic which is a drug or herb used to improve the heart health [30]. A single serving of Moringa seeds has almost three times the amount of iron as spinach. This is especially important for vegetarians and vegans and those who suffer from iron deficiency anaemia [31]. Moringa seeds are beneficial for diabetes and blood sugar regulation. The scientists believe that the compounds which help in lowering the blood sugar level are isothiocyanates [32].

Fibre is an important factor for the maintenance of healthy cardiovascular system [33]. Moringa seeds are rich in soluble fibre, so that, it can help in maintaining a healthy digestive system [34]. Studies show that Moringa seed powder has an anti – proliferative effect on the breast cancer cell. [35]. Iron deficiency will lead to anaemia. Moringa seeds are high in iron. Moringa seeds can be taken as a substitution for iron supplements [36]. A Study done on experimental rats showed that the Moringa seed extract given twice daily had healed liver injury induced with liver fibrosis over the course of eight weeks [37]. The process by which the dormant embryo wakes up, grow out of the seed coat and establishes itself as a seedling is called germination [30].

Research revealed that the water extracts of *M. oleifera* seed kernel exhibited significant free radical scavenging activity. Thus, the study shows that the water extract possessed potent antioxidant activity which supports the use of *M. oleifera* seed kernel as a natural antioxidant [38]. Different parts of the plant such as bark, leaves, seeds, flowers, roots, and immature pods and mature pods contains large number of important bioactive constituents such as terpenoids, alkaloids, tannins, steroidal aglycones and reducing sugars [39]. The active components found in the seeds in the Aqueous and Hydro-alcoholic extract of *M. oleifera* seeds are Methionine, cysteine, 4-(alpha-L-rhamnopyranosyloxy) benzylglucosinolate, Moringine, benzylglucosinolate, niazimiciniazirin [4].

Hence, germinating and fermenting the Moringa seeds could be a viable approach to increase the nutritional benefits which would reduce hidden hunger and other health complications

#### V. LIMITATIONS OF THE STUDY

- 1) All the phytochemicals could not quantify due to unavailability of reagents, cumbersomeness of the process and time constraints.
- 2) Quantification of all the micro nutrients was not done due to time and financial constraints.
- 3) There are various methods to assess antioxidant potential of the plant samples. However, in the present study, only preferred methods were used to analyse each of the activities. These assessments can also be carried out by various other methods which can provide more accuracy to the result.

#### VI. PROSPECTS FOR FUTURE RESEARCH

- 1) A study on the other plant parts of *M. oleifera* can be done.
- 2) The cancer prevention potential of raw, germinated and fermented *M. oleifera* seeds against various cancer types can be analysed.
- 3) Antifungal and anti-inflammatory activities of raw, germinated and fermented *M. oleifera* seeds can also be studied.
- 4) A study on *M. oleifera* seed oil can be done.

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